CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Azafenidin

Chemical Code # 5025, Tolerance # 52130
SB 950: not assigned

Original date 8/9/99
Revised 9/7/00

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, **possible adverse effect**
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, **possible adverse effect**
Oncogenicity, mouse:	No data gap, **possible adverse effect**
Reproduction, rat:	No data gap, **possible adverse effect**
Teratology, rat:	No data gap, **possible adverse effect**
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	No data gap, no adverse effect

Toxicology one-liners are attached.

All record numbers for the above study types through 176251 (Document no. 52130-177) were examined. This includes all relevant studies indexed by DPR as of 9/7/00.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T000907
Duncan, 9/7/00.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

****52130-112 156353** "Combined Chronic Toxicity/Oncogenicity Study with DPX-R6447-50 Two-Year Feeding Study in Rats" (Malley, L. 835-E. I. du Pont de Nemours and Company, Haskell Laboratory, Elkton Road, Newark, Delaware, Study HLR 941-96, 3/24/97). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was given in the diet daily to 92 Crl:CD®BR rats/sex/dose (including 10 rats/sex/dose for 1-year interim sacrifice) at levels of 0, 5, 15, 30, 300 and 900 ppm for 24 months. Although 5 deaths (3 males, 2 females) were attributed to the test substance in the 900 ppm dose group with jaundice and/or thrombosis reported in 4 of 5 and liver necrosis and porphyrin deposits were noted in 5 of 5, there were no significant differences in rat survival at any dose level. Females receiving 900 ppm diets had transiently lower body weight during the first year of the study, corresponding to slightly lower food consumption during the first 3 months. Hematologic changes consistent with anemia (i.e., minimally decreased Hb and Ht) occurred during most of periods tested at 900 ppm; erythrocytic changes were characterized by microcytosis and hypochromasia (decreased MCV and MCH). Organ weight effects consisted of increased liver weights at terminal sacrifice in high-dose males and in 300 and 900 ppm females. Multinucleate hepatocytes and hepatocellular cytomegaly were seen at 300 and 900 ppm females. At terminal sacrifice, increased hemosiderin pigment deposition in the liver and kidneys, porphyrin pigment deposition in the liver, and bone marrow hyperplasia were noted in high-dose males and females. The total hepatic cytochrome P-450 content in male rats was significantly less than controls at the 1-week (at 300 and 900 ppm) and also reduced at 1-year. **Possible Adverse Effect:** increased thyroid follicular cell adenoma in 900 ppm males only. For nonneoplastic effects**, NOEL (M/F)=30 ppm** (M: 1.21 mg/kg/day; F: 1.58 mg/kg/day based on hepatocellular changes in females and cytochrome P-450 inhibition in males). **Acceptable.** Kellner, 5/3/99.

52130-113 156354 addendum to 52130-112 156353 "Combined Chronic Toxicity/Oncogenicity Study with DPX-R6447-50 Two-Year Feeding Study in Rats" (Makovec, G., 835-E. I. du Pont de Nemours and Company, Haskell Laboratory, Elkton Road, Newark, Delaware, Study HLR 941-96 Supplement No. 1, 4/17/97). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was given in the diet daily to 92 Crl:CD®BR rats/sex/dose (including 10 rats/sex/dose for 1-year interim sacrifice) at levels of 0, 5, 15, 30, 300 and 900 ppm for 24 months. This supplement to the rat combined chronic toxicity/oncogenicity study contained photomicrographs of representative sections of major organs, examples of the most common neoplasms in males and females and examples of findings considered to be test compound-related. This record had no new data or interpretations and was a **supplemental submission,** to satisfy MAFF Japanese requirements. Kellner, 5/11/99.

52130-147 157595 addendum to 52130-112 156353 "Combined Chronic Toxicity/Oncogenicity Study with DPX-R6447-50 Two-Year Feeding Study in Rats" (O=Connor, J. 835-E. I. du Pont de Nemours and Company, Haskell Laboratory, Elkton Road, Newark, Delaware, Study HLR 941-96 Supplement No. 2, 6/26/97). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was given in the diet daily to 92 Crl:CD®BR rats/sex/dose (including 10 rats/sex/dose for 1-year interim sacrifice) at levels of 0, 5, 15, 30, 300 and 900 ppm for 24 months; a supplemental study to measure thyroid hormone levels (T_3, T_4, and TSH) was initiated because of an increase in thyroid follicular cell adenomas in 900 ppm males. Approximately 3 months after initiation of dosing, blood samples were collected from 10 randomly selected males/group at 0, 5, 15, 50, 300, or 900 ppm and thyroid hormone levels were measured using commercially available radio-immunoassay (RIA) kits. Serum T_3 levels were significantly decreased (66%, 77% and 71% of control in males at 15, 300 and 900 ppm, respectively). No effects on T_3 levels were seen at 5 and 50 ppm and no effects on serum concentrations of T_4 or TSH were observed at any concentration of DPX-R6447-50. A lack of TSH hypersecretion was cited as evidence that the increased thyroid follicular cell adenomas may not be compound-related. Although TSH hypersecretion (frequently reported in cases of thyroid tumorigenesis) was lacking, these data were not considered sufficiently conclusive to warrant a change in the evaluation of thyroid tumors seen in combined

rat study 52130-112:156353 (i.e., timing of hormone measurements did not correspond to the reporting of increased thyroid tumors at terminal sacrifice); this study is a **supplemental submission.** Kellner, 5/11/99.

CHRONIC TOXICITY, DOG

52130-108 156349 "Chronic Toxicity Study with DPX-R6447-50, One Year Feeding Study in Dogs" (Mertens, J. 831-WIL Research Laboratories, Inc. Ashland, Ohio. Study HLO 924-96, 3/26/97). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was administered orally (via the feed) to 5 Beagle dogs/sex/dose at levels of 0, 5, 10, 30, 120 and 360 ppm for 52 weeks. There were no unscheduled deaths and no effects on body weight, food consumption or clinical signs. High-dose males had decreased erythrocyte count, hemoglobin and hematocrit at week 51; males at this level also showed decreased mean activated partial thromboplastin time (APTT) at week 12 and 25. Increases in group mean alanine transferase (ALT) at 30, 120 and 360 ppm and in alkaline phosphatase (ALP) at 120 and 360 ppm were seen in males and females during most of the study. Microscopic changes observed in the liver included minimal to mild hepatocyte enlargement at 30 ppm and above. Multiple hepatocyte nuclei, usually in the enlarged hepatocytes, were noted in most dogs (8 of 10) at 30 ppm and all dogs at 120 and 360 ppm. Cytoplasmic pigment (probably porphyrin) was seen in centrilobular and midzonal hepatocytes and Kupffer cells of two males and one female at 5 ppm, four males and four females at 10 ppm and all animals at 30 ppm and above. **No Adverse Effects. LOEL(M/F) = 5 ppm (males: 0.13 mg/kg/day; females: 0.16 mg/kg/day based on cytoplasmic pigment in hepatocytes and Kupffer cells in all dose groups without histopathology or enzyme leakage). Acceptable. Kellner, 4/13/99.

52130-109 156350 addendum to 52130-108 156349 "Chronic Toxicity Study with DPX-R6447-50, One Year Feeding Study in Dogs" (Mertens, J. 831-WIL Research Laboratories, Inc. Ashland, Ohio. Study HLO 924-96, 3/26/97). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was administered orally (via the feed) to 5 Beagle dogs/sex/dose at levels of 0, 5, 10, 30, 120 and 360 ppm for 52 weeks This supplement to the chronic toxicity study contained photomicrographs of liver showing three compound-related effects (i.e., porphyrin pigment, hepatocellular enlargement and multiple nuclei) in addition to photos of kidneys, lungs, heart and spleen from control and treated dogs. This record had no new data or interpretations and was a **supplemental submission,** to satisfy MAFF Japanese requirements. Kellner, 4/21/99.

ONCOGENICITY, RAT

See Combined Toxicity, Rat.

ONCOGENICITY, MOUSE

****52130-110 156351** "Oncogenicity Study with DPX-R6447-50 Eighteen-Month Feeding Study in Mice" (Cox, L. 832-E. I. du Pont de Nemours and Company, Haskell Laboratory, Elkton Road, Newark, Delaware, Study HLR 284-96, 11/27/96). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was given in the diet daily to 80 Crl:CD®-1(ICR)BR mice/sex/dose at 0, 10, 30, 300 or 900 ppm for 18 months. Significantly lower body weights were seen in 900 ppm females beginning at day 49; by day 553, mean body weight in this group was 5.2% lower than control. Body weight gain was also significantly reduced in this group (19.2% lower during the initial 13 weeks of the test); from test day 91-553, body weight gain in female mice was comparable across all dose groups. Clinical signs included slightly increased incidence of hyperreactivity and a significantly increased incidence of perineal masses in the 900 ppm males. Changes in MCV or MCH at the 3-, 6-, or 12-month sampling times were noted in the high-dose animals, but were not evident at 18-months. Increases in absolute and/or relative liver weight were noted in high-dose males and females and in 300 ppm females. **Possible Adverse Neoplastic Effects:** increased incidence of liver masses were noted in 900 ppm males (14/60 vs. 7/60, $p=0.074$) corresponding with statistically significant increases in multiple hepatocellular adenomas (3/60 vs. 0/60, $p<0.05$) and hemangiosarcomas (2/60 vs. 0/60, $p<0.05$) using the Cochran-Armitage trend test but not by group comparisons. Non-neoplastic liver findings consisted of small, but significant increases in

apoptosis in high-dose males, deposition of brown, granular pigment (i.e., porphyrin) in Kupffer cells (300 and 900 ppm males and females) and significant increases in eosinophilic foci of cellular alteration in the livers of males at 900 ppm and females at 300 and 900 ppm. For non-neoplastic effects, **NOEL(M/F)=30 ppm** (M: 4.0 mg/kg/day; F: 5.1 mg/kg/day based on liver findings of porphyrin in Kupffer cells and eosinophilic foci of cellular alteration in the livers of males at 900 ppm and females at 300 and 900 ppm and increases in hepatic cell proliferation at 300 and 900 ppm in males). **Acceptable.** Kellner, 4/19/99.

REPRODUCTION, RAT

**** 060 149624**, "Reproductive and Fertility Effects with DPX-R6447-49/-50 Multigeneration Reproduction Study in Rats", (Kim H. Kreckmann, 834, E.I. DuPont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE., Report # HLR 646-94, 30 August 1995). 30 Crl:CD®BR rats per sex per group received DPX-R6447 (Batch numbers DPX-R6447-49 with 96.7% purity and DPX-R6447-50 with 98.6% purity) at doses of 0, 5, 30, 180, and 1080 ppm in the diet through 2 generations with one litter per generation. Parental animals began receiving treated feed 70 days prior to mating in the first generation and 105 days pre-mating in the second. The incidence of diarrhea was increased for F1 males and both F1 sexes had lower bodyweights early in premating at 180 ppm. Parental NOEL = 30 ppm [F0 (M) 1.7 mg/kg, (F) 2.31 mg/kg, F1 (M) 2.33 and (F) 2.80 mg/kg] (Lower bodyweight early in premating period of F1 parents and increased incidence of diarrhea in F1 males at 180 ppm). **Possible Adverse reproductive effects:** Decreased implantation efficiency, increased gestation length, decreased litter size and lower pup weight; Reproductive NOEL = 30 ppm (Decreased implantation efficiency with decreased litter size at 180 ppm, increased gestation length, clinical observations in F1a and F2a pups at 180 ppm including lower bodyweight at day 0). **Acceptable**. (H. Green and J. Gee, 6/16/97).

TERATOLOGY, RAT

**** 058 149622**, "Developmental Toxicity of DPX-R6447-50 in Rats", (Susan M. Munley, 833, E.I. DuPont de Nemours & Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, Newark, DE., Report # HLR 1-95, 11 July 1995). 25 mated Crl:CD ®BR female rats per group received 0 (methyl cellulose), 3, 8, 16, and 24 mg/kg/day of DPX-6447 (98.6% purity, batch number DPX-6447-50) by gavage on gestation days 7 through 16 (confirmation of copulation was designated gestation day 1). Reduced maternal bodyweight gain was reported at 24 mg/kg/day (may be due to developmental toxicity, see discussion in section VI.B.). **Possible adverse effects: Increased resorptions and reduced litter size were noted at 16 and 24 mg/kg/day**. Additionally, lower fetal weights were recorded at 24 mg/kg/day. Developmental NOEL = 8 mg/kg/day. **Acceptable.** (H. Green and J. Gee, 6/16/97).

52130-114 156355 addendum to 52130-058 149622 "Developmental Toxicity of DPX-R6447-50 in Rats" (Munley, S. 833-E. I. du Pont de Nemours and Company, Haskell Laboratory, Elkton Road, Newark, Delaware, Study HLR I-95 Supplement No. 1, 4/18/97). DPX-R6447-50 technical (Batch DPX-R6447-50, purity of 98.6%) was given by oral gavage to 25 mated Crl:CD®BR rats/dose at levels of 0, 3, 8, 16 and 24 mg/kg/day on gestation days 7 through 16. This record had no new data or interpretations and was a **supplemental submission,** to address concerns expressed by the U.S. EPA which focused on the high variability (e.g., coefficient of variation as high as 26%) in the homogeneity of some of the dosing suspensions during initial stages of the study (first 4 days of dosing). From day 5 to the end of the dosing period, the mixing methodology was improved and acceptable homogeneity was achieved; no effect on the experimental outcome was anticipated. Kellner, 5/12/99.

**** 151; 164114**; "A Dermal Prenatal Developmental Toxicity Study of Azafenidin Technical in Rats" (J.L. Schardein, WIL Research Laboratories, Inc., Ashland, OH, Project ID # HLO - 1998-01717, 9/2/98). Azafenidin Technical (H-20690, 98.6% purity) in 0.5% aqueous methylcellulose (0.5% MC),

was administered dermally to groups of 25 Crl:CD(SD)BR rats once daily from gestation days 6 through 19. Dosage levels of 0 (0.5% MC), 5, 25, 50 and 100 mg/kg/day were administered at a dose volume of 2 ml/kg. All animals were exposed to the test material for 6 hours/day. One female in the 25 mg/kg/day group died on gestation day 7. No compound-related clinical signs or dermal irritation were observed. Mean gravid uterine weights in the 50 and 100 mg/kg/day dose levels were significantly reduced (64.6 g and 49.3 g vs. 83.7, respectively; $p < 0.05$), whereas the mean net body weight and mean net body weight gain in the treated groups were comparable to those of the control group. This result suggests that the reduced mean body weight gains are attributed to increased total resorption **[Possible adverse effects:** 23% and 41.3% vs. 5.4%, respectively, $p < 0.05$] and retarded fetal growth as demonstrated by the reduced fetal weights (3.1 g and 2.6 g vs. 3.8 g, respectively; $p < 0.01$) at 50 and 100 mg/kg/day dose levels. Reduced fetal weight and total resorptions were also reported at 25 mg/kg/day. Dose-related increase in skeletal malformations (bent limb bones) was reported in 25, 50 and 100 mg/kg/day groups. Maternal NOEL = 100 mg/kg (no treatment-related effects at HDT); developmental NOEL = 5 mg/kg (skeletal malformations, decreased fetal weight). **Acceptable** (Leung, 11/20/98).

TERATOLOGY, RABBIT

** 059 149623, "Developmental Toxicity Study of DPX-R6447-50 in Rabbits", (Susan M. Munley, 833, E.I. DuPont de Nemours & Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, Newark, DE., Report # HLR 67-95, 2 June 1995). 20 mated Hra: (NZW)SPF female rabbits per group received DPX-R6447-50 technical (98.6% purity, batch # DPX-R6447-50) at doses of 0 (0.5% methyl cellulose), 12, 36, 100, and 300 mg/kg/day by gavage on gestation days 7 through 19 (gestation day 0 was the day of mating). At the high dose level, two does were found dead (gestation day 17), thirteen aborted, and 1 litter was totally resorbed . Maternal NOEL = 100 mg/kg/day. Note: high dose data were not evaluated due to excess maternal toxicity. **Developmental toxicity is not indicated**. Developmental NOEL = 100 mg/kg/day. **Acceptable**. (H. Green and J. Gee, 6/13/97).

GENE MUTATION

** 062 149626, "Mutagenicity Testing of DPX-R6447-50 in the Salmonella Typhimurium and Escherichia Coli Plate Incorporation Assay", (Kathy M. Gerber, B.A., 842, E. I. DuPont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE., Report # HLR 55-95, 7 June 1995). *Salmonella typhimurium* strains TA97a, TA98, TA100, and TA1535 and *Escherichia coli* strain WP2uvrA (pKM101) were exposed in triplicate to azafenidin (azafenidin technical, Batch number DPX-R6447-50, 98.6% purity) concentrations of 0, 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate for 48 hours in the presence and absence of activation in two trials. The positive controls were functional. **An increase in the reversion rate is not indicated at treatment levels up to 5000 µg/plate. Acceptable**. (H. Green and J. Gee, 6/12/97).

** 066 149630, "CHO/HGPRT Mutation Assay with DPX-R6447-50 with Confirmation", (Richard H. C. San and Jane J. Clarke, 842, Microbiological Associates, Inc., Rockville, MD., Report # HLO 475-95, 6 October 1995). Chinese hamster ovary (CHO) cells were exposed in duplicate to azafenidin technical (98.6% purity, Batch number DPX-R6447-50) concentrations of 0, 75, 125, 250, 500, and 750 µg/ml for 5 hours at 37 ± 1°C. in the presence and absence of activation. A precipitate formed in cultures at 750 µg/ml in both the initial and the confirmatory mutagenesis assays. Duplicate cultures per concentration ± S9. Expression period was 7-9 days followed by replating with 5 dishes/per initial culture for mutation frequency. **An increase in forward mutations is not indicated**. **Acceptable**. (H. Green and J. Gee, 6/13/97).

52130-115 156356 "IN-G6286-2: Mutagenicity Testing in the *Salmonella typhimurium* and *Escherichia coli* Plate Incorportation Assay" (Mathison, B. 842-E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Study #HLR 829-96, 5/7/97). IN-G6286-2 (Batch IN-G6286-2, purity 100%) was tested for mutagenic potential in the *Salmonella, E. coli*/Mammalian-Microsome Mutagenicity Assay at levels of 0, 10, 50, 100, 500, 1000, 2500 and 5000 ug/plate (triplicate plating) using *Salmonella* strains TA100, TA97a, TA98, TA1535 and *E. coli* strain WP2 *uvrA* with and without metabolic activation (Aroclor 1254-induced rat liver S-9 fraction) in two trials. All colony counts indicated that the test article was negative for mutagenicity. **Supplemental Study** (test substance is a metabolite of Azafenidin) Kellner, 513/99.

CHROMOSOME EFFECTS

** 064 149628, "Mouse Bone Marrow Micronucleus Assay of DPX-R6447-50", (Kathy M. Gerber, B. A., 843, E. I. DuPont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE., Report # HLR 443-95, 17 July 1995). 5 or 6 Crl:CD®-1 mice per sex per group received azafenidin technical (98.6% purity, Batch # DPX-R6447-50) at doses of 0, 2500, or 5000 mg/kg by gavage. Bone marrow was sampled 24, 48, or 72 hours after treatment. The proportion of PCEs per 1000 erythrocytes was depressed in treated animals. **An increase in micronuclei frequency is not indicated. Acceptable.** (H. Green and J. Gee, 6/12/97).

** 065 149629, *"In Vitro* Mammalian Cytogenetic Test of DPX-R6447-50 Using Human Peripheral Lymphocytes", (Patrick T. Curry, Ph.D., 843, Microbiological Associates, Inc., Rockville, MD., Report # HLO 474-95, 4 October 1995). Human adult male peripheral blood lymphocytes were exposed in duplicate to azafenidin technical (98.6% purity, Batch number DPX-R6447-50) at concentrations of untreated, 0, 88, 175, 350, and 700 µg/ml for 4 hours in the presence and absence of activation. Two trials with duplicate cultures for each trial per concentration were used and 100 metaphases per replicate culture were scored for aberrations. The mitotic index was decreased as a function of concentration. **An increase in the frequency of chromosomal aberrations is not indicated. Acceptable**. (H. Green and J. Gee, 6/12/97).

DNA DAMAGE

** 063 149627, "Assessment of DPX-R6447-50 in the In Vitro Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes", (Karin S. Bentley, Ph. D., 844, E. I. DuPont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE., Report # HLR 419-95, 30 June 1995). Primary rat hepatocytes (from young adult male Crl:CD®BR rats) were exposed to azafenidin technical (98.6% purity, Batch number DPX-R6447-50) concentrations of 0, 1, 5, 10, 50, 100, 150, 200, and 250 µg/ml for 18 hours in 2 trials. UDS was measured by autoradiography with ^{3}H-Thymidine. **An increase in unscheduled DNA synthesis is not indicated under conditions of the assay. Acceptable.** (H. Green and J. Gee, 6/12/97).

NEUROTOXICITY

** 052; 149616; "Acute Oral Neurotoxicity Study of DPX-R6447-50 Rats" (Mikles, K.A., E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Report No. HLR 753-95, 3/29/96). 818. DPX-R6447-50 Technical (Batch number DPX-R6447-50, purity=98.6%), prepared as a suspension in 0.5% aqueous methylcellulose, was administered by gavage in a single dose at concentrations of 0, 100, 300, or 900 mg/kg to 12 Crl:CD®BR rats per sex per dose level. No animals died. Discolored urine was observed in both male and female animals at the 300 (6 animals per sex) and 900 (12 animals per sex) mg/kg dose levels. Motor activity measurements revealed a statistically significant decrease in both mean total duration of movements and mean total number of movements in both males and females at 300 and 900 mg/kg. There were no compound-related gross or microscopic lesions observed in the central or peripheral nervous system. **No adverse effects. NOEL (M/F)=100 mg/kg (based on clinical signs and decreased motor activity). Acceptable**. (Corlett, 5/13/97)

** 057; 149621; "Subchronic Oral Neurotoxicity Study of DPX-R6447-50 Rats" (Malley, L.A., E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Report No. HLR 87-96, 6/25/96). 827. DPX-R6447-50 Technical (Batch number DPX-R6447-50, purity=98.6%) was admixed to the feed at concentrations of 0, 50, 750, or 1500 ppm and fed to 12 Crl:CD®BR rats per sex per dose level daily for a period of 13 weeks. There were no treatment-related mortalities. Red stained cageboard in both males and females at 1500 ppm and pallor associated with eyes and/or ears in both males and females at 1500 ppm and in females at 750 ppm were observed. A statistically significant increase in pale eyes in both males and females at 1500 ppm during week 4 and week 8 was observed during FOB assessments. Motor activity measurements revealed a statistically significant decrease in mean total duration of movements in females at 750 and 1500 ppm during week 4. There were no compound-related gross or microscopic lesions observed in the central or peripheral nervous system. NOEL (M)=44.1 mg/kg/day (750 ppm, based on FOB endpoints), NOEL (F)= 3.80 mg/kg/day (50 ppm, based on clinical signs and decreased total mean duration of movements in motor activity assessment) and NOAEL (M)=95.1 mg/kg/day and NOAEL (F)=108 mg/kg/day (both based on no adverse effects). **(No adverse effects)**. **Acceptable**. (Corlett, 5/19/97)

SUBCHRONIC STUDIES

(Oral)

054, 107; 149618, 156348; "Subchronic Oral Toxicity: 90-Day Study with DPX-R6447-19 Feeding and One-Generation Reproduction Study in Rats" (Malley, L.A., 821-E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Report No. HLR 568-94, 3/28/95; Revision 1, 4/18/97 [The report was revised to the change the NOAEL to 300 ppm based on a change in understanding of the biological importance of decreased P-450]). DPX-R6447 (Batch number DPX-R6447-19 Technical, purity=98.7%) was admixed to the feed at concentrations of 0, 50, 300, 900, or 1500 ppm and fed to 10 Crl:CD®BR rats per sex per dose level daily for a period of 90 days. One control female and three 1500 ppm female animals designated to the subchronic feeding study died during the study. A decrease in mean body weights in both males and females at 1500 ppm on days 28, 56, and 91 was observed. Pallor was observed in both males and females at 1500 ppm. Statistically significant decreases in hemoglobin and hematocrit were observed in both males and females at 900 and 1500 ppm. Statistically significant increases in reticulocytes and methemoglobin were observed in both males and females at 900 and 1500 ppm. Statistically significant decreases in red blood cells and statistically significant increases in white blood cells were observed in both males and females at 1500 ppm. Microscopic examination revealed dose-related incidences of pigment in hepatic Kupffer cells, increased extramedullary erythropoiesis in the spleen, and increased erythropoiesis in the bone marrow at 900 and 1500 ppm in both males and females. A dose-related decrease in cytochrome P-450 content was observed at 300, 900, and 1500 ppm in both males (65%, 59%, and 49% of control, respectively, p=0.05) and females (60%, 35%, and 31% of control, respectively, p=0.05). **No adverse effects**. NOEL=50 ppm (M)=4.02 mg/kg/day and (F)=4.73 mg/kg/day (both based on reduction in cytochrome P-450 activity). NOEL has been revised from 300 ppm (hematology and histopathology) to 50 ppm. **Acceptable**. Corlett, 5/28/97; updated, Kellner, 5/7/99.

054; 149618; "Subchronic Oral Toxicity: 90-Day Study with DPX-R6447-19 Feeding and One-Generation Reproduction Study in Rats" (Malley, L.A., E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Report No. HLR 568-94, 3/28/95). DPX-R6447 (Batch number DPX-R6447-19 Technical, purity=98.7%) was admixed to the feed at concentrations of 0, 50, 300, or 900 ppm, fed to 10 Crl:CD®BR rats per sex per dose level daily for a period of 90 days, and then used to initiate a one-generation, one-litter reproduction study. Each female was continually housed with a male from the same dose group until evidence of copulation was obtained. Exophthalmus was observed in 3 animals at 900 ppm. Statistically significant decrease in mean body weight gain was observed during gestation at 300 and 900 ppm. No litters were produced in any female in either the 300 or 900 ppm groups. **Possible adverse effect indicated:** no progeny produced. NOEL (reproductive)= NOAEL =5.43 mg/kg/day (no progeny produced).

NOEL (maternal)=50 ppm or 5.43 mg/kg (based on decrease in mean body weight gain) **Supplemental study** (only one generation used and no necropsies or histopathology performed). (Corlett, 5/29/97)

053, 106 149617 156347 "Subchronic Oral Toxicity: 90-Day Study with DPX-R6447-19 Feeding Study in Mice" (Malley, L.A., 821-E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Report No. HLR 783-92, 3/30/95; Revision No. 1: 4/18/97 [Revision addressed a change in the review of the biological significance of porphyrin pigment in the liver by the registrant with a change in NOAEL(M) to 300 ppm]). DPX-R6447 (Batch number DPX-R6447-19 Technical, purity=98.7%) was admixed to the feed at concentrations of 0, 50, 300, 900, or 1500 ppm and fed to 10 Crl:CD®-1(ICR)BR mice per sex per dose level daily for a period of 90 days. No animals assigned to the subchronic feeding study died during the study. Brown-colored urine and brown stained cageboard were observed in both males and females at 1500 ppm. Statistically significant decreases in hemoglobin and hematocrit were observed in both males and females at 900 and 1500 ppm. Dose-related increases in mean relative liver weights in both males and females at 900 and 1500 ppm were observed. Microscopic examination of the liver revealed dose-related incidences of centrilobular hypertrophy (at 900 and 1500 ppm in males and at 1500 ppm in females) and brown, granular pigment (at 300, 900, and 1500 ppm in males and at 900 and 1500 ppm in females). **No adverse effects**. NOEL (M)=7.94 mg/kg/day (50 ppm) and (F)=65.8 mg/kg/day (300 ppm) (both based on dose-related incidences of brown, granular pigment in the liver) **Acceptable**. (Corlett, 6/2/97). Updated (Kellner, 5/25/99).

055; 149619; "Subchronic Oral Toxicity: 90-Day Study with DPX-R6447-50 Feeding Study in Dogs" (Tompkins, E.C., WIL Research Laboratories, Inc., Ashland, OH, Project ID: WIL-189012, 10/4/95). 821. DPX-R6447-50 (Batch number DPX-R6447-50 (technical), purity=98.6%) was admixed to the feed at concentrations of 0, 10, 60, 120, or 240 ppm and fed to 4 beagle dogs per sex per dose level daily for a period of 13 weeks. No animals died during the study. No treatment-related clinical signs were observed. Statistically significant increases in serum alanine aminotransferase levels were observed in both males and females at 240 ppm during both week 6 and week 12. Microscopic examination of the liver revealed enlarged hepatocytes and statistically significant incidences of increased cytoplasmic pigment in both males and females at 60, 120 and 240 ppm. **No adverse effects**. NOEL (M)=0.34 mg/kg/day and (F)=0.33 mg/kg/day (based on enlarged hepatocytes and increased cytoplasmic pigment in the liver). **Acceptable**. (Corlett, 6/9/97)

100, -117; 149682, 156358 ; "A 13-Week Mechanistic Study with DPX-R6447-50 in Dogs" (Mertens, J.J.W.M., WIL Research Laboratories, Inc., Ashland, OH, Project ID: WIL-189018, 5/24/96). DPX-R6447-50 (Batch number DPX-R6447-50 (technical), purity=98.6%) was admixed to the feed at 0 (8M/4F), 1 (4M), 10 (4M), 240 (4M), or 900 (4M/4F) ppm and fed to beagle dogs for a period of 13 weeks. A satellite group (4 males each at 0 and 240 ppm) was included to establish the reversibility of hepatic porphyrin accumulation. No deaths or treatment-related clinical signs were noted. Significant increases in mean serum alanine aminotransferase (ALT) and alkaline phosphatase (AP) were seen at 240 ppm (males) and 900 ppm (females) throughout the study. Significant increases in mean ALT were not seen in the 240 ppm recovery group after day 42. Significant increases in mean serum gamma glutamyl transferase values were observed at 900 ppm in females at day 35 and 90. Significant increase in mean relative liver weight was observed in both sexes at 900 ppm. Liver necrosis, endogenous pigment that polarized light, and minimal to moderate cytomegaly was noted at 900 ppm (both sexes). **No adverse effects**. NOEL (M)=0.362 mg/kg/day (based on based on the absence of changes in serum chemistry parameters). NOEL (F) cannot be determined. **Supplemental study** (0 and 900 ppm female dose groups only). (Corlett, 6/11/97); DPX-R6447-50 causes a reversible accumulation of protoporphyrin in the liver of male dogs; accumulation of porphyrin will result in mild hepatocyte injury, as evidenced by increased levels of serum ALT and AP. Based on porphyrin accumulation, inhibition of protoporphyrinogen oxidase occurs at levels of 10, 240 and 900 ppm, but not 1 ppm in male dogs. Reversible decreases in P-450s (CYPs, 1A2 and 2E1) were also noted at 240 ppm (7.93-8.24 mg/kg/day). Based on decreased body weight gain (females only), increased serum chemistry parameters, and morphologic evidence of hepatocellular injury progressing to necrosis at 900 ppm (29.5 and 28.1 mg/kg/day in males and females, respectively) 900 ppm was

considered in excess of the MTD. Updated (Kellner, 5/20/99).

52130-176; 174841; "Subchronic Oral Toxicity: 28-Day Study with IN-G6286, Feeding Study in Rats"; (G.S. Ladics; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; Project ID: DuPont-2928; 8/30/99); Ten Crl:CD (SD) IGS BR rats/sex/group were treated in the diet with 0, 70, 200, 1400 or 7500 ppm of IN-G6286 Technical (photolytic decomposition product of azafenidin) (purity: 93.5%) for 28 days ((M): 0, 5.42, 16.0, 108.5, 570.4 mg/kg/day, (F) 0, 6.02, 16.6, 114.6, 641.8 mg/kg/day). No mortality nor treatment-related clinical signs resulted from the treatment. The target organ was the liver. In the clinical chemistry evaluation, the mean sorbitol dehydrogenase activity was increased for the 7500 ppm group males ($p<0.05$). Mean serum cholesterol levels were minimally elevated for both sexes in the 7500 ppm treatment group ($p<0.05$). The mean absolute liver weights for the males in the 7500 ppm treatment group and the mean relative liver/body weight values for both sexes in the 7500 ppm group were increased ($p<0.05$). Histopathological examination of the liver revealed minimal centrilobular hypertrophy for the males in the 1400 (2/10) and 7500 (10/10) ppm treatment groups and for the females in the 7500 (8/10) ppm treatment group. **No adverse effect indicated. NOEL:** (M) 200 ppm (15.98 mg/kg/day) (based upon the hepatic hypertrophy noted for the males in the 1400 ppm treatment group), (F) 1400 ppm (114.6 mg/kg/day) (based upon the hepatic hypertrophy and increased relative liver weight noted for the 7500 ppm treatment group). Hepatocellular hypertrophy, an adaptive response to the test material, was not considered to be toxicologically significant. **Study supplemental** (non-guideline study). (Moore, 5/23/00)

177; 176251; "IN-G6286: Subchronic Toxicity 90-Day Feeding Study in Rats", (G. S. Ladics; Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; Lab Report No. DuPont-3386; 6/11/00); IN-G6286 (photolytic decomposition product of azafenidin; purity = 99.78%) was fed in the diet at concentrations of 0, 200, 1400, 6000, and 12000 ppm (0, 11, 76, 323, or 633 mg/kg/day in males and 0, 13, 89, 386, or 761 mg/kg/day in females) to groups of 10M/10F Crl:CD rats for 90 days; no deaths; signs of toxicity consisted of black ocular discharge and decreased duration of movement in the neurobehavioral test battery in high dose rats; reduced food consumption and body weight gain in 1400, 6000, and 12000 ppm males and 12000 ppm females; increased liver weight relative to body weight in 6000 and 12000 ppm males and 12000 ppm females which correlated histologically with hepatocellular centrilobular hypertrophy; no adverse effects; NOEL (M) = 1400 ppm, (F) = 6000 ppm based on increased relative liver weight, and hepatocellular hypertrophy; Supplemental. (Duncan, 9/7/00)

(Dermal)

056; 149620; "Repeated Dose Dermal Toxicity: 28-Day Study with DPX-R6447-50 in Rats" (Filliben, T.A., E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Report No. HLR 587-96, 8/26/96). 822. DPX-R6447-50 Technical (Batch number DPX-R6447-50, purity=98.6%), moistened with deionized water, was applied to the shaved skin of 5 Crl:CD® BR rats per sex per dose level at concentrations of 0, 80, 400, 1000 mg/kg for 6 hours daily for 28 days. No animals died. No treatment-related clinical signs were observed in any test animal. No erythema or edema was observed in any test animal. Macroscopic and microscopic examinations of the test animals revealed no treatment-related abnormalities. **No adverse effects.** NOEL (systemic and dermal, M/F)=1000 mg/kg **. Acceptable**. (Corlett, 6/12/97)

METABOLISM STUDIES

52130-116 156357 "^{14}C-DPX-R6447: Metabolism Study in the Rat" (Fasano, W. 851- E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Study# HLR 250-96, 4/17/97). DPX-R6447-50 (Technical grade, 98.8% purity), [triazolyl-iminium-^{14}C]DPX-R6447 (sample #21027 and 21465, spec. activity of 54.7 and 56.7 uCi/mg, respectively, purity >98%) or [phenyl(U)-^{14}C]DPX-R6447 (sample #21010, spec. activity of

59.7 uCi/mg, purity of 97.7%) in PEG 400 was given to 5 Crl:CD® BR rats/sex/dose for blood level and metabolite studies or 4/sex/dose for tissue distribution studies) at 5 mg/kg (low dose) or 75 mg/kg (high dose); additional low- and high-dose groups with 3 rats sex/dose had cannulated bile ducts to measure excretion via bile. Maximum plasma concentrations of total radioactivity (C_{max}) were higher in females (4.173 ug/g versus 2.592 ug/g for males at low dose); the time needed to reach maximum plasma levels (tC_{max}) occurred earlier in males (1.2 hr) than in females (3.2 hr) at 5 mg/kg. AUC was 2-fold greater in females, suggesting increased bioavailability in females. Terminal elimination half-life in plasma (approximately 40 hr) showed no sex, dose level, or route-related effects. In males, residue levels associated with red blood cells showed a slower rate of elimination (t_2 = 75 hr) and was prolonged in the high dose females (t_2 = 107 hr). At the low dose 55% of radiolabel was in the urine and 40% in the feces, mostly during 48 hr postdose; at the high-dose, amounts in urine and feces were almost equal. Tissue residues were low and usually contained less than 1% of the dose at sacrifice. Metabolism was primarily accomplished by *O*-dealkylation yielding G6044 followed by hydroxylation and conjugation with glucuronic acid or sulfate. Metabolites in urine were primarily glucuronide conjugates of KQ959, KT416, TX799 and G6044. Males eliminated approximately 37% of the radiolabel in bile over the 24-hr collection period at either the 5 or 75 mg/kg dose level. Metabolites eliminated in bile were identified as sulfate conjugates of KQ959, KT416, TX799 and G6044 and glucuronide conjugates of KT976 and TX217. **Unacceptable**, but upgradeable with multidose group and complete I.V. dose group. Kellner, 5/20/99.

52130-165; 170046; "^{14}C-DPX-R6447: Metabolism Study in the Rat" (W.J. Fasano, E.I. du Pont de Nemours & Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, Study # HLR 250-96, Supplement No. 1, 11/12/97). Groups of 10 Crl:CD BR rats/sex were pretreated with 14 daily oral doses of nonlabeled DPX-R6447 (Batch # DPX-R6447-24, 98.8 - 99.4% purity) followed by a single oral dose of ^{14}C-DPX-R6447 at 5 (13.43 - 18.75 uCi/mg, 18.4 - 20.3 uCi/rat) or 75 mg/kg (males only, SA = 0.81 uCi/mg, 20.1 uCi/rat). The dosing vehicle was PEG 400). A majority of the eliminated radioactivity was recovered in the urine (47.6 - 55.1%) with a smaller portion in the feces (38.5 - 43.4%). Less than 0.5% of the administered dose was detected in tissues at terminal sacrifice (168 hrs). For the most part, HPLC-radio- chomatographic profiles of excreta were similar to those obtained from the non-conditioned, single-dose studies (see Document 52130 -116, record # 156357). However, three additional metabolites not previously characterized in the single-dose studies (< than 5% of the administered dose) were subsequently isolated and identified as KQ959-sulfate, dihydroxylated-R6447(B), and KQ963. Total cytochrome p450 were similar to vehicle controls in both sexes at 5 mg/kg/day dose level. In contrast, total cytochrome p450 was significantly reduced in male rats at 75 mg/kg/day dose level. This result is consistent with the mode of action of azafenidin (i.e., inhibition of protoporphyringen oxidase activity and ultimately, heme synthesis). **Supplemental.** (Leung, 8/6/99).

Collectively, data from both studies satisfies the current requirements for an acceptable animal metabolism study.